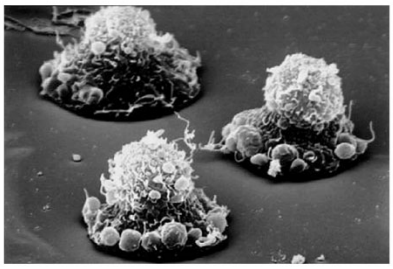
Part A

Multiple Choice Questions: (0.5 mark each for a total of 5 marks)

1. The following image of epithelial cells undergoing apoptosis was taken with which instrument or technique?



a. Light microscope.
b. Scanning electron microscope.
c. X-ray crystallography.
d. Transmission electron microscope.
e. Either b or d.

2. Which of the following reversibly binds GTP?

a. Actin
b. α-tubulin
c. β-tubulin
d. Myosin II
e. b and c

3. What is the last event that occurs immediately before microtubules fall apart during catastrophe?

a. The availability of tubulin in the cytosol falls below the critical concentration.
b. Hydrolysis of ATP to ADP and loss of the ATP cap.
c. Hydrolysis of GTP to GDP and loss of the GTP cap.
d. Conformational change of protofilaments into a curved shape.
e. Rescue.

4. Which of the following is <u>false</u>?

a. Thymosin sequesters actin and prevents growth of actin filaments.
b. Profilin competes with thymosin and promotes assembly of actin filaments.
c. Stathmin prevents dynamic instability of microtubules.
d. α-actinin provides enough space for myosin bundles to slide between actin filaments.
e. Filimin is an example of a gel-forming protein.

5. Which of the following is <u>not</u> a characteristic of kinesins?

a. They spend approximately 5% of their time in the attached state.
b. They form attachment with microtubules.
c. They take processive steps.
d. They exhibit weak forces during movement (less than 5 pN).
e. Binding is induced by ATP.

6. Which of the following best describe(s) the role of Ca^{2+} in the contraction of striated muscle?

a. Ca^{2+} is released from the Golgi apparatus.
b. Ca^{2+} binds to tropomodulin and induces a conformational change.
c. Ca^{2+} binds to troponin C and induces a conformational change.
d. Movement of tropomyosin exposes binding sites on actin filaments.
e. Both c and d.

7. What is a protein kinase?

a. An enzyme capable of breaking down any protein.
b. A protein that specifically induces degradation of cyclins.
c. Any protein that is bound to a kinase.
d. An enzyme that removes a phosphate from a target protein.
e. An enzyme that mediates the transfer of a phosphate to a target protein.

8. Let us state, arbitrarily, that a single cell cycle begins at the start of M-phase and ends at end of G_2. In this model, which of the following choices indicates a series of events in the order in which they would occur?

a. Increased levels of S-cyclin; anaphase; Cdc25 activates M-Cdk.
b. Degradation of securin; elevation of S-cyclin; DNA replication.
c. Completion of DNA replication; S-Cdk triggers S-phase; APC activation.
d. DNA replication; elevation of G_1/S-cyclin, Cdc25 activates M-Cdk.
e. None of the above.

9. A “normal” cell can undergo cell cycle arrest or apoptosis to deal with the potentially damaging effects of abnormal mitogenic stimulation. How does this occur?

a. Excessive Myc production produces a protein that binds to Mdm2 and stabilizes p53.
b. Phosphorylation of Bad leads to activation of Bcl-2, which in turn induces apoptosis.
c. Mitosis stimulates Bax directly, leading to the subsequent release of cytochrome c.
d. Binding of PDGF to a receptor stimulates Myc production and the cell enters S-phase.
e. None of the above.

10. Which of the following best describe(s) the role of caspases during apoptosis?

a. They cleave intracellular proteins at an aspartate site.
b. They cleave focal adhesion kinase, leading to disruption of cell adhesion.
c. They cleave lamins, causing disassembly of the nucleus.
d. They cleave filaments of the cytoskeleton, causing changes in cell shape.
e. All of the above are correct.

Part B

Long Answer Questions (5 marks each for a total of 20 marks).

1. Describe the important principles of confocal microscopy, and list the advantages over other techniques that use light as an energy source.

- "Confocal" refers to the equidistance between light source and object, and object and detector.
- There are 2 pinholes: one that focuses light on a single point in the specimen (producing and "optical section", and one that focuses the image on the detector. Both pinholes effectively reduce stray light.
- Utilizes fluorescence (i.e. filters and fluorophores e.g. FITC) and high energy lasers (e.g. He-Ne and Ar).

Advantages

- Clear images several microns into tissue are possible. They are useful for thick tissue specimens.
- 3D reconstructions are possible.
- Don't have to cut sections.
- Reduced background signal.

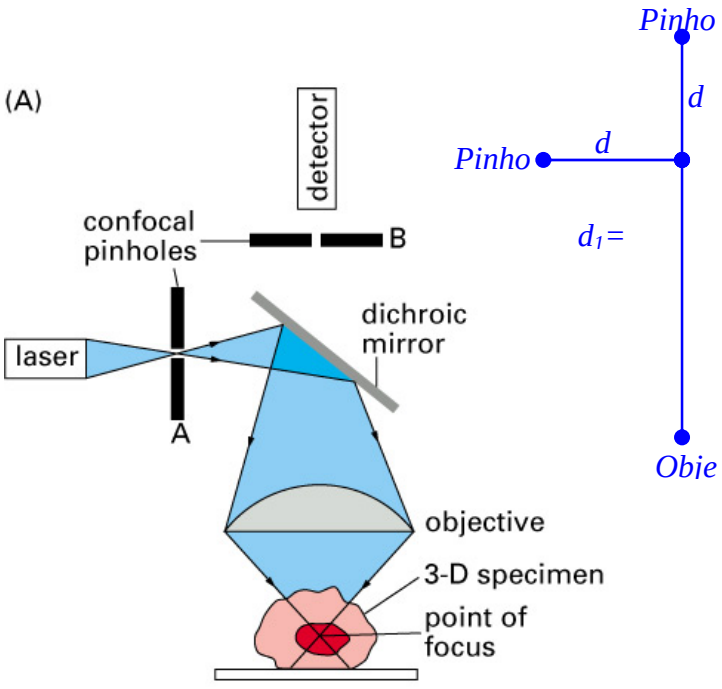


Figure 9–18 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

2. Describe the process of treadmilling in actin filaments, and give 2 examples of accessory proteins and how they regulate this process.

Process

- Treadmilling is basically the process of addition and removal of subunits to actin filaments in which there is no net change in length.
- Initially, when the concentration of ATP and actin subunits is high (i.e. above Cc, the critical concentration), subunits are added at both the plus (“+” or “T”) and minus (“-“ or “D”) end.
- As the concentration of subunits and ATP is reduced, and becomes more limiting, subunit addition will occur mostly at the plus end. (This is because addition of subunits here is thermally more likely because a conformational change is not required, as at the minus end. It may also be explained that the Cc for the “T” end is lower than the Cc for the D end, and so addition at the T end occurs at lower subunit concentrations. See Fig 9-46b)
- Eventually, subunit and ATP concentrations will drop below Cc and addition of subunits to the minus end is not likely (we assume that it does not occur). Then, subunits are lost at the minus end.
- This gives us a state of stability in which there is no net growth or shrinkage, i.e. “treadmilling”.

Possible Examples (*only the first 2 will be marked, 0.5 marks each*).

- Thymosin sequesters actin and prevents addition to actin filaments.
- Profilin recruits actin and promotes growth.
- Arp complex (Arp2/3) reduces subunit loss (or “caps”) at the minus end during formation of actin filament branches.
- Cofilin destabilizes actin filaments and leads to loss of subunits (i.e. increases turnover).
- Tropomyosin will stabilize filament (e.g. in muscle) and reduce treadmilling.
- CapZ is a capping protein that binds to the plus end and reduces subunit addition.
- Tropomodulin is a capping protein that binds to the minus end and reduces subunit loss.

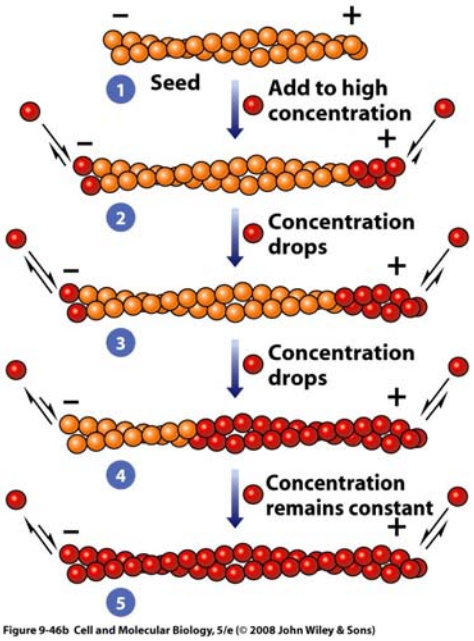


Figure 9-46b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

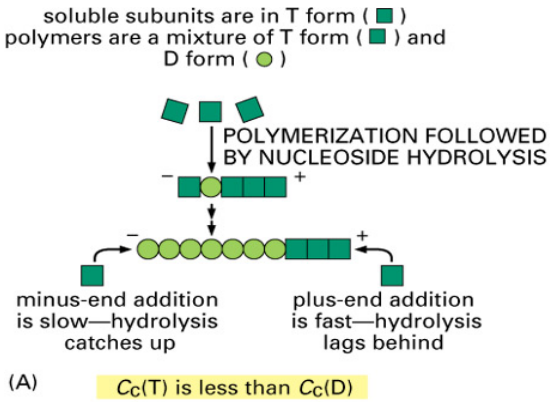


Figure 16–9. Molecular Biology of the Cell, 4th Edition.

3. A cell is currently in the first growth phase (G_1) of the cell cycle and damaged DNA has been detected. Describe what will happen to the cell and explain the process.

- Generally, the cell cycle is arrested in G_1 phase at the DNA damage checkpoint and will not proceed into S-phase, where DNA is normally replicated.
- After DNA damage is "sensed" a protein kinase is activated.
- This induces phosphorylation of p53 and dissociation of Mdm2 away from p53.
- p53 is then stabilized (i.e. active).
- p53 is a gene regulatory protein that will bind to the *p21* gene, which will induce transcription of p21 mRNA and translation (synthesis) of the protein p21, a Cdk inhibitor (or CKI).
- p21 will bind to a Cdk-cyclin complex (or active Cdk etc.) causing Cdk inhibition. p21 (like all CKIs) binds to both the Cdk and cyclin component of the complex and interferes with catalytic activity.
- So, in this specific case, G_1/S-cyclin and S-cyclin are inhibited. Therefore, the cell will be arrested in G_1 phase and will not proceed to S-phase.

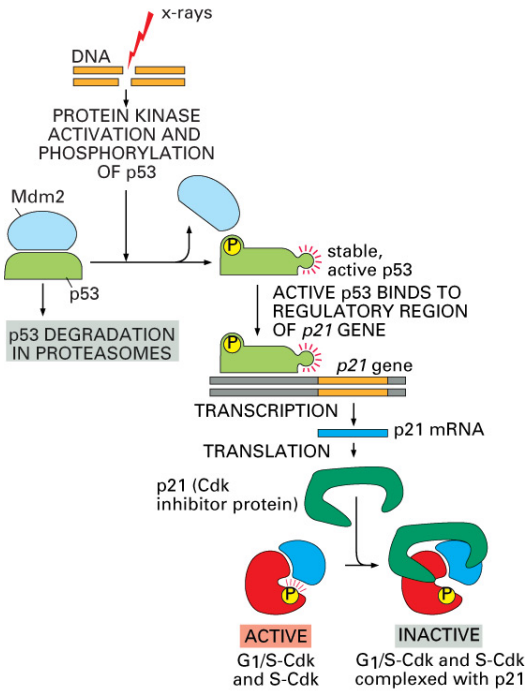


Figure 17–33. Molecular Biology of the Cell, 4th Edition.

4. The immune system relies on apoptotic mechanisms to eliminate foreign or diseased cells. Name and describe this pathway of apoptosis.

This question refers specifically to the *extrinsic pathway* of apoptosis, and this must be clear in the answer. No marks will be given for information regarding the intrinsic pathway.

No more than *1 mark* awared for information regarding cleavage of other intracellular proteins (such as FAK, lamins, or cytoskeleton etc.) or loss of membrane asymmetry, since this question focuses primarily on the *extrinsic pathway* of apoptosis.

- The basics of this mechanism include activation of procaspases from outside of the cell.
- An extracellular signal, the Fas ligand, from a killer lymphocyte (killer T cell, Tc, lymphocyte also acceptable) binds to the Fas death receptor at the target cell.
- Both ligand and receptor are transmembrane proteins.
- Activation of the death receptor recruits adaptor proteins, called FADD (Fas-activated death domain), that mediate recruitment of procaspases to form DISC (death-induced signalling complex).
- Thus, many procaspases are located adjacent to one another and will cleave each other at aspartate sites.
- This cleavage will lead to active caspases and the caspase cascade.